JOURNAL OF BONE AND MINERAL RESEARCH
Volume 20, Number 11, 2005
Published online on July 18, 2005; doi: 10.1359/JBMR.050718

Fibroblast Growth Factor-23 Is Regulated by 1α,25-Dihydroxyvitamin D

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ABSTRACT: Serum FGF-23 regulation was studied in patients with hypoparathyroidism or pseudohypoparathyroidism treated with calcitriol. Serum FGF-23 levels changed in parallel in response to changes in serum 1,25-D, suggesting that FGF-23 may be regulated by 1,25-D. In addition, the phosphaturic effect of FGF-23 may be diminished in the absence of PTH action on the kidney.

Introduction: Fibroblast growth factor (FGF)-23 is a recently described hormone that has been shown to be involved in the regulation of phosphate and vitamin D metabolism. The physiologic role of FGF-23 in mineral metabolism and how serum FGF-23 levels are regulated have yet to be elucidated. Three patients with mineral metabolism defects that allowed for the investigation of the regulation of FGF-23 were studied.
Materials and Methods: Patient 1 had postsurgical hypoparathyroidism and Munchausen's syndrome and consumed a pharmacologic dose of calcitriol. Patient 2 had postsurgical hypoparathyroidism and fibrous dysplasia of bone. She was treated with increasing doses of calcitriol followed by synthetic PTH(1-34). Patient 3 had pseudohypoparathyroidism type 1B and tertiary hyperparathyroidism. She underwent parathyroidectomy, which was followed by the development of hungry bone syndrome and hypocalcemia, requiring treatment with calcitriol. Serum FGF-23 and serum and urine levels of mineral metabolites were measured in all three patients.
Results: Patient 1 had an acute and marked increase in serum FGF-23 (70 to 670 RU/ml; normal range, 18–108 RU/ml) within 24 h in response to high-dose calcitriol administration. Patient 2 showed stepwise increases in serum FGF-23 from 117 to 824 RU/ml in response to increasing serum levels of 1α,25-dihydroxyvitamin D (1,25-D). Finally, before parathyroidectomy, while hypercalcemic, euphosphatemic, with low levels of 1,25-D (10 pg/ml; normal range, 22–67 pg/ml), and with very high serum PTH (863.7 pg/ml; normal range, 6.0–40.0 pg/ml), patient 3 had high serum FGF-23 levels (217 RU/ml). After surgery, while hypocalcemic, euphosphatemic, and with high serum levels of serum 1,25-D (140 pg/ml), FGF-23 levels were higher than preoperative levels (305 RU/ml). It seemed that the phosphaturic effect of FGF-23 was diminished in the absence of PTH or a PTH effect.
Conclusions: Serum FGF-23 may be regulated by serum 1,25-D, and its phosphaturic effect may be less in the absence of PTH.
J Bone Miner Res 2005;20:1944–1950. Published online on July 18, 2005; doi: 10.1359/JBMR.050718

Key words: fibroblast growth factor-23, fibrous dysplasia, McCune-Albright syndrome, hypoparathyroidism, pseudohypoparathyroidism, phosphate, phosphorus, 1α-hydroxylase, vitamin D, phosphaturia

INTRODUCTION

The first evidence for the existence of a phosphate-regulating hormone (phosphatonin) was the resolution of phosphaturia and hypophosphatemia with the excision of small mesenchymal tumors, which presumably elaborated such a factor.[1,2] Several proteins including fibroblast growth factor-23 (FGF-23), matrix extracellular phosphoglycoprotein (MEPE), frizzle related protein-4 (FRP-4), and fibroblast growth factor-7 (FGF-7) have been shown to be overexpressed in phosphaturic tumors.[3,4] To date, the preponderance of the data suggests that FGF-23 is the most physiologically relevant.[5] The identification of mutations in FGF-23 in patients with autosomal dominant hypophosphatemic rickets suggested that FGF-23 was a phosphate-regulating hormone.[6] Subsequently, FGF-23 was found to

The authors have no conflict of interest.

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be highly expressed in tumors that cause tumor induced osteomalacia (TIO)[7] and elevated in the serum of patients with the phosphaturic syndromes of TIO, X-linked hypophosphatemia (XLH),[8] and fibrous dysplasia of bone.[8,9] In addition, missense mutations in FGF-23 have been identified in familial tumoral calcinosis, which is characterized by decreased urinary excretion of phosphorus, hyperphosphatemia, and ectopic calcification.[10,11]

In addition to inducing renal phosphate wasting and causing hypophosphatemia, elevations in serum FGF-23 result in frankly or inappropriately low levels of serum 1α,25-dihydroxyvitamin D (1,25-D) through the inhibition of renal 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase).[12] The ability of FGF-23 to directly induce renal phosphate wasting and inhibit 1α-hydroxylase was confirmed in mouse models in which FGF-23 was overexpressed or knocked out.[13–15] Robust levels of FGF-23 are measurable in the serum of normal humans, with bone being a likely physiologic source of circulating FGF-23,[9,16,17] although by RT-PCR analysis, some level of FGF-23 expression has also shown in heart, liver, thyroid/parathyroid, small intestine, testis, and skeletal muscle.[6] Phosphate and calcitriol in rodents[16,18,19] and phosphate in humans[20,21] have been suggested as regulators of serum FGF-23.

Because changes in serum phosphorus and calcium usually parallel changes in serum 1,25-D, it has been difficult to ascertain their unique effects on serum FGF-23 in normal human physiology. Furthermore, the study of FGF-23 physiology is complicated by the overlapping effects of FGF-23 and PTH. Both have similar effects on renal proximal tubule phosphate handling but opposing effects on 1α-hydroxylase activity. We have overcome the confounding effect of PTH by studying FGF-23 metabolism in three patients with defects in either PTH secretion or action. Patient 1 had postsurgical hypoparathyroidism and Munchausen's syndrome and consumed a pharmacological dose of calcitriol. Patient 2 had postsurgical hypoparathyroidism and the FGF-23 hypersecretory state of fibrous dysplasia. Fibrous dysplasia is caused by somatic activating mutations of the G protein α-subunit, $G_s\alpha$, in bone marrow stromal cells, and results in ligand-independent signaling of the cAMP pathway.[22] She was treated with escalating doses of calcitriol (1,25-D), followed by two fixed doses of PTH. Patient 3 had pseudohypoparathyroidism type 1B (PHP1B) and tertiary hyperparathyroidism. Patients with PHP1B have an imprinting defect in the $G_s\alpha$ encoding gene *GNAS*, which leads to a tissue-specific $G_s\alpha$ deficiency in renal proximal tubules and PTH resistance in this tissue. As a result there is little conversion of 25-hydroxyvitamin-D to 1,25-D, low levels of serum 1,25-D, higher than normal reabsorption of filtered phosphorus, and high serum phosphorus levels. In PHP1B, bone is sensitive to the effects of PTH. Our results show that increases in serum 1,25-D are associated with increases in serum FGF-23 and suggest that, despite high FGF-23 levels, in the absence of a PTH effect, the ability of FGF-23 to induce phosphaturia may be diminished.

MATERIALS AND METHODS

Patient 1

Patient 1 was a 39-year-old-woman with postsurgical hypoparathyroidism. Her hypoparathyroidism had been difficult to manage, presumably because of malabsorption. Studies to evaluate malabsorption were not diagnostic. She had been hospitalized >12 times in the preceding year for symptomatic hypocalcemia, which required intravenous calcium administration. Her psychosocial situation was complicated and stressful. Her reported medical regimen before admission included 35 μg of calcitriol per day divided t.i.d., 10 g of calcium carbonate per day divided b.i.d., teriparatide [PTH(1-34); Lilly, Indianapolis, IN, USA) 20 μg t.i.d., hydrochlorothiazide (50 mg/day), and 125 μg/day levothyroxine. On admission to the NIH Clinical Center, her laboratory values were as follows: phosphorus, 5.8 mg/dl (normal range, 2.5–4.8 mg/dl); calcium, 1.75 mM (normal range, 2.05–2.50 mM); creatinine clearance, 134 ml/min (normal range, 90–125 ml/min); PTH, 15.3 pg/ml (normal range, 6.0–40.0 pg/ml); 25-hydroxyvitamin-D, 7 ng/ml (normal range, 10–68 ng/ml); 1,25-D, 26 pg/ml (normal range, 22–67 pg/ml); thyroid-stimulating hormone (TSH), 0.11 μIU/ml (normal range, 0.40–4.00 μIU/ml).

She was prescribed calcitriol 10 μg every 8 h and calcium carbonate 2500 mg every 12 h. On the third day of admission, her nurse insisted she take the first dose of the day of 10 μg of calcitriol and 2500 mg of calcium carbonate under observation. She did not receive teriparatide. The next morning, her serum calcium was found to be 2.80 mM. All medications were held. Munchausen's syndrome was suspected and eventually confirmed with the assistance of a consultation by the psychiatry service. In light of the diagnosis of Munchausen's syndrome, it is not clear what medications she had been taking before admission or on the first 2 days of hospitalization. Serum for 1,25-D measurement was available on days 2, 3, 4, 5, and 8 only.

Patient 2

Patient 2 was a 20-year-old woman with McCune-Albright syndrome (fibrous dysplasia of bone, café-au-lait skin pigmentation, precocious puberty, and hyperthyroidism).[23] Two years before the current evaluation, she had undergone subtotal thyroidectomy elsewhere for hyperthyroidism, at which time she was inadvertently rendered hypoparathyroid. Medical treatment on admission included calcium carbonate 2500 mg b.i.d., calcitriol 0.5 μg t.i.d, and levothyroxine 112 μg/day. At the time of admission laboratory values were as follows: total serum calcium, 1.74 mM; phosphorus, 5.2 mg/dl; PTH, 13 pg/ml; 25-hydroxyvitamin-D, 45 ng/ml; 1,25-D, 57 pg/ml; TSH, 1.37 μIU/ml; creatinine clearance, 119 ml/min.

Patient 2 was treated with calcitriol and calcium from days 0 to 9. The calcitriol dose was as follows: days 1–3, 0.75 μg every 6 h, days 4–6, 1.00 μg every 6 h, days 7 and 8, 1.25 μg every 6 h, and day 9, 0.75 μg every 6 h. As calcitriol was withdrawn on day 9, PTH treatment was initiated. Teriparatide [lsqb]PTH(1-34)[rsqb], 20 μg every 12 h was added on days 9–11, and the dose was increased to 20 μg

Table 1. Serial Serum Values and Calcitriol Treatment for Patient 3

Day number	*FGF-23 (18–108 RU/ml)**	*Phosphorus (1.5–4.8 mg/dl)*	*1,25-D (22–67 pg/ml)*	*Ionized Ca (1.17–1.31 mM)*	*Alkaline phosphatase (37–116 U/liter)*	*Calcitriol dose (μg/day)*
Preoperative	215	3.0	10	1.47	579	0
1	106	2.6		1.09	557	0.5
2		2.5		1.18	560	2
3		2.6		1.02	599	2
4		3.2		1.20	704	4
5		3.2		1.19	794	4
6	158	3.5		1.19	761	8
7	123	3.6		1.21	810	8
8	305	3.8	140	1.15	761	8

Reference ranges are shown in column heads.
* The normal range for FGF-23 represents the mean ± SD.[19]

every 8 h for days 12–24. The calcium carbonate dose was initially 6250 mg every 8 h and tapered down to 2500 mg every 8 h on day 6.

Patient 3

Patient 3 was a 47-year-old woman with familial PHP1B, characterized by renal resistance to PTH without Albright hereditary osteodystrophy. The diagnosis was confirmed by demonstration of loss of imprinting of *GNAS* exon 1A and the presence of a *STX16* deletion mutation that has been shown to be associated with familial PHP1B.[24] Because of inadequate medical therapy, she developed autonomous parathyroid hyperfunction with an elevated serum PTH and calcium. Before surgery, her laboratory values were as follows: ionized calcium, 1.47 mM (normal range, 1.17–1.31 mM); phosphorus, 3.4 mg/dl; PTH 863.7, pg/ml; creatinine, 0.8 mg/dl (normal range, 0.7–1.3 mg/dl); 25-hydroxyvitamin-D, 16 ng/ml; 1,25-D, 10 pg/ml. She had evidence of hyperparathyroid bone disease, with an elevated serum alkaline phosphatase of 571 U/liter (normal range, 37–116 U/liter) and subperiosteal resorption of the digits on radiographs.

The patient underwent a neck exploration, during which a single parathyroid adenoma was removed. Intraoperative PTH decreased from a baseline value of 570.0 to 51.7 pg/ml at 20 minutes after resection. Over the next several days, she developed hungry bone syndrome as evidenced by symptoms of hypocalcemia, a decrease in both serum calcium and phosphorus, a further increase in alkaline phosphatase, and a rise in serum PTH from 51.7 to 183.0 pg/ml (Table 1). Symptomatic hypocalcemia was initially treated with intravenous calcium gluconate, and subsequently with oral calcium carbonate, up to 2500 mg every 6 h and calcitriol. The calcitriol doses on postoperative days 1, 2, 4, and 6 onward were as follows: 0.5 μg/day, 1.0 μg every 12 h, 1.0 μg every 6 h, and 2.0 μg every 6 h, respectively.

All patients gave informed consent and were studied under NIH Institutional Review Board–approved protocols for the study of hypoparathyroidism, fibrous dysplasia, or hyperparathyroidism.

Methods

Blood was drawn in the morning in a fasting state at approximately the same time that second morning voided urine specimens were collected. Laboratory testing of serum constituents of mineral metabolism and other hormones was performed with standardized commercial assays. Tubular maximum of phosphate per glomerular filtration rate (TmP/GFR) was assessed according to the method of Bijvoet et al.[25] FGF-23 was measured using a commercially available C-terminal assay (Immutopics, San Clemente, CA, USA). Normative data on serum FGF-23 levels have been previously reported (63.2 ± 44.9 [SD] RU/ml).[9]

RESULTS

In both hypoparathyroid patients (patients 1 and 2), serum FGF-23 increased within 24 h in parallel in response to changes in serum 1,25-D (Figs. 1A and 2A). FGF-23 increased almost 10-fold (70 to 670 RU/ml) in patient 1 and 7-fold (117 to 824 RU/ml) in patient 2. Serum phosphorus and TmP/GFR decreased in response to increases in serum FGF-23, but remained frankly or relatively elevated in the absence of PTH (Figs. 1B and 2B; days 1–9). Serum calcium increased from 1.75 to 2.36 mM in patient 1 (Fig. 1C) and 1.74 to 2.56 mM in patient 2 (Fig. 2C). In patient 2, serum FGF-23 increased immediately, whereas serum calcium did not increase until day 4 (Fig. 2C). In addition, in patient 2 there was a second peak in serum FGF-23 on day 9 that coincided with a second peak in serum 1,25-D, without coincident changes in serum calcium or phosphorus. This occurred when serum levels of FGF-23 were almost 10-fold above the upper limit of the normal range. In patient 2, the best fit lines plotted for the relationship between serum FGF-23 and 1,25-D during the calcitriol and PTH treatment phases show that the dose–response curve of FGF-23–1,25-D was shifted upward and to the left in the presence of a fixed dose of PTH, suggesting a much greater response in FGF-23 production in the presence of PTH (Fig. 2D). This phenomenon did not occur when the relationships between FGF-23 and calcium were analyzed separately during the calcitriol and PTH treatment phases.

In patient 3, serum FGF-23 levels were elevated in the presence of hypercalcemia (216 RU/ml), high serum PTH (863.7 pg/ml), and low levels of serum 1,25-D (10 pg/ml; Table 1). Postoperatively, during a period of hypocalcemia (ionized calcium, 1.15 mM) and elevated serum 1,25-D lev-

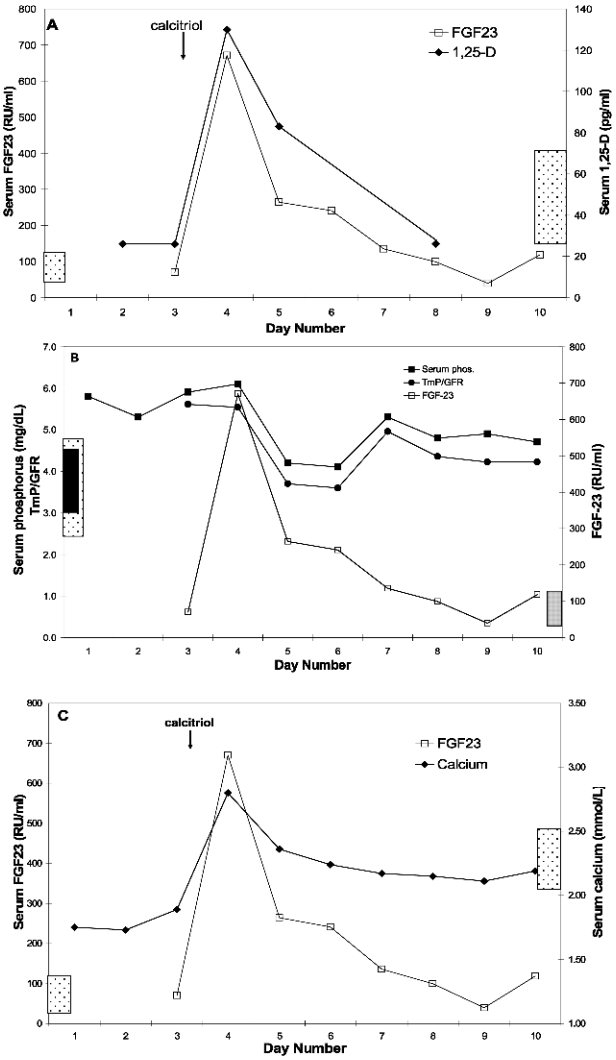

FIG. 1. Changes in serum FGF-23, 1,25-D, phosphorus, TmP/GFR, and calcium in response to high-dose calcitriol treatment in patient 1. Serial serum values of (A) FGF-23 and 1,25-D, (B) FGF-23, phosphorus, and TmP/GFR, and (C) FGF-23 and calcium are shown. The normal ranges are indicated by the stippled boxes on the assigned *y* axis. The normal range for TmP/GFR is indicated by the solid box. The normal range for FGF-23 represents mean ± SD.[9] A dose of 10 μg of calcitriol was administered on day 3. The bolus of 1,25-D stimulated a rapid and dramatic increase in serum FGF-23 and calcium.

els (140.0 pg/ml), serum FGF-23 was even more elevated than during the hypercalcemic phase (305 versus 215 RU/ml; Table 1). As in patients 1 and 2, serum phosphorus was in the normal range at both time-points, despite high serum FGF-23 (Table 1). Serum for measurement of FGF-23 was only available preoperatively and on postoperative days 1, 6, 7, and 8, and serum for 1,25-D was only available preoperatively and on postoperative day 8.

DISCUSSION

Serum FGF-23 levels changed in parallel in response to changes in serum 1,25-D in patients 1 and 2 with postsurgical hypoparathyroidism (Figs. 1A and 2A). Serum FGF-23, in patient 3 with pseudohypoparathyroidism, was elevated at a time when calcium and PTH were elevated, but 1,25-D was low. However, similar to the other patients, serum FGF-23 levels rose in response to calcitriol administration and high levels of serum 1,25-D for treatment of hypocalcemia (Table 1). In patient 2, who was treated with calcitriol followed by PTH, the response of FGF-23 to changes in 1,25-D was greater in the presence of PTH (Fig. 2D). In all three patients with a diminished PTH effect on the kidney, very high levels of FGF-23 (levels similar to that seen in tumor-induced osteomalacia) were not associated with low serum phosphorus or TmP/GFR, suggesting PTH may be necessary for the full phosphaturic effect of FGF-23. This is especially evident in patient 2, who despite >7 days of very high FGF-23 levels, maintained serum phosphorus and TmP/GFR in the high normal range. It was not until the addition of PTH to the regimen on day 9 that serum phosphorus and TmP/GFR fell to the low normal or frankly low range. Taken together, these data suggest serum 1,25-D may be involved in regulating serum FGF-23 and suggest that the phosphaturic effect of FGF-23 may, at least in part, be dependent on the presence of PTH. When these data are considered in the context of the established evidence that FGF-23 can decrease the production of 1,25-D, they suggest that a classic endocrine feedback loop between FGF-23 and 1,25-D may exist. The purpose of this counter-regulation, as well as the ability of FGF-23 to lower serum phosphorus, may be to protect against both 1,25-D–induced hypercalcemia and/or the development of a high serum phosphate–calcium solubility product, and thus ectopic calcification.

FGF-23 was initially identified as a phosphatonin, and its effect on vitamin D metabolism, in terms of lowering serum 1,25-D, whereas prominent, was not consistently observed, and thus a central role in vitamin D metabolism was not emphasized. This may be because of the relatively high rate of secondary hyperparathyroidism observed in phosphate wasting states such as TIO, which would tend to counter the effects of FGF-23 on renal 1α-hydroxylase.[26] Several recent studies in rodents showed a more central role of FGF-23 in vitamin D metabolism. FGF-23 decreases renal 1α-hydroxylase activity and increases renal 25-hydroxy vitamin D-24-hydroxylase activity, both of which ultimately result in a decrease in serum 1,25-D.[12,14,15] In addition, similar to what we show here in humans, a direct stimulatory effect of 1,25-D on serum FGF-23 levels in rodents[18,19] and in vitro[27] has been shown recently. Furthermore, in the rodent studies, again as we show here in humans, serum phosphorus remained elevated in the face of high 1,25-D and FGF-23. We propose that the inability of FGF-23 to induce phosphaturia sufficient to normalize serum phosphorus in the rodent studies may be caused by hypoparathyroidism. PTH levels were not reported in the rodent studies, but they were most likely low in the setting of 1,25-D–induced hypercalcemia. Furthermore, we have shown previously in hyperphosphatemic patients with hypoparathyroidism and normal renal function that high serum FGF-23 levels were unable to stimulate phosphate excretion.[20] The implication is that PTH is necessary for the full phosphaturic action

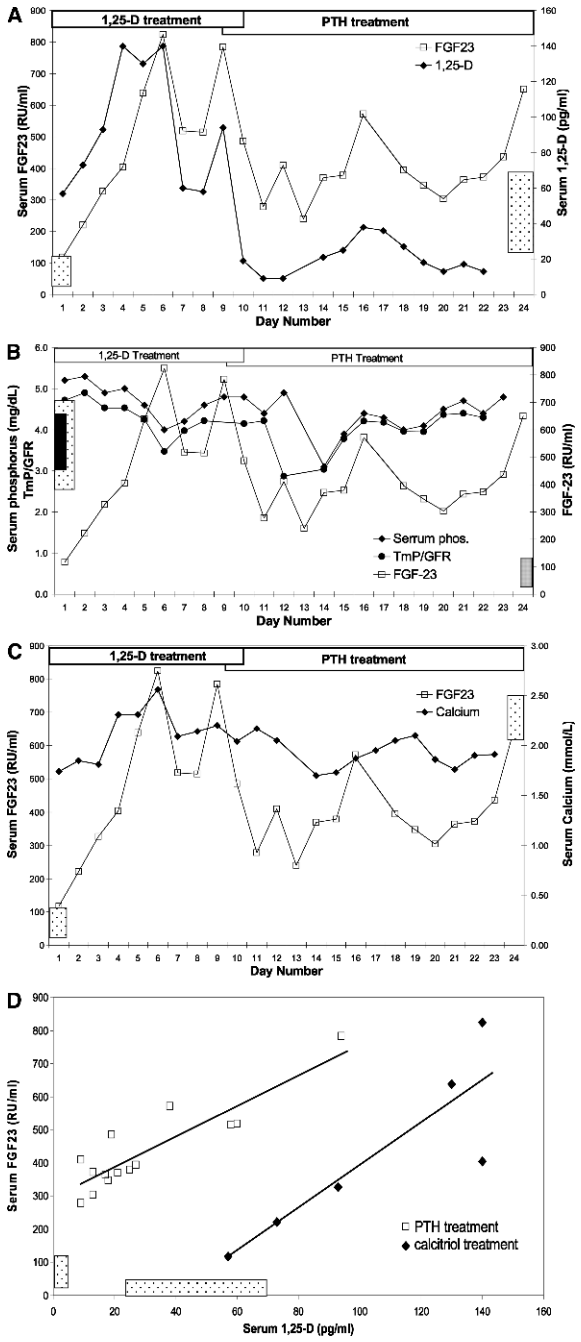

FIG. 2. Changes in serum FGF-23, 1,25-D, phosphorus, TmP/GFR, and calcium, and the relationship between FGF-23 and 1,25-D in response to treatment with calcitriol and PTH in patient 2. Serial serum values of (A) FGF-23 and 1,25-D, (B) FGF-23, phosphorus, and TmP/GFR, and (C) FGF-23 and calcium and are shown. The normal ranges are indicated by the stippled boxes on that assigned indicated axes. The normal range for TmP/GFR is indicated by the solid box. The normal range for FGF-23 represents mean ± SD.[9] The patient was treated with escalating, then tapering, doses of calcitriol followed by treatment with PTH as indicated. The calcitriol and PTH treatment periods are indicated by the labeled areas at the top. (D) The relationship between FGF-23 and 1,25-D during the calcitriol and PTH treatment phases are indicated by the best fit lines during the respective treatment. FGF-23 and calcium levels increased within 24 h after 1,25-D administration, and the relationship of FGF-23–1,25-D was shifted in the presence of PTH.

of FGF-23. In renal proximal tubule cells, the presence of the sodium/phosphate transporter 2a (NPT2a) in the brush border is necessary for phosphorus reabsorption.[28] Whereas FGF-23 is capable of decreasing NPT2a message and protein levels,[15,29] PTH is required for the retrieval of NPT2a from the cell surface.[28] It is therefore possible that, in the absence of sufficient PTH action at the kidney, NPT2a remains on the cell surface and continues to promote phosphate reabsorption.

The degree to which FGF-23 is regulated by phosphorus in humans is less clear. In one published report in intact humans, the authors were unable to show an effect on FGF-23 by either phosphate loading or deprivation.[30] However, in a more recent report, phosphate deprivation and loading decreased and increased serum FGF-23, respectively.[21] In other studies performed in subjects with impaired renal function, it is difficult to discern whether elevations in FGF-23 were the direct effect of elevated serum phosphorus or accumulation of biologically active FGF-23 caused by renal insufficiency.[31,32] There are convincing data in rats showing that changes in serum phosphorus can bring about changes in serum FGF-23, independent of the degree of renal insufficiency.[19] However, in that same model in which hyperphosphatemic rats with renal insufficiency and mildly elevated serum FGF-23 levels were treated with 1,25-D, 1,25-D was able to dramatically increase serum FGF-23 above the levels brought about by high serum phosphorus.[19] Those data also suggest a dominant role for 1,25-D, relative to phosphorus, in the regulation of serum FGF-23.

Studies of patients with hyperparathyroidism have shown conflicting results with regard to the correlation between serum FGF-23 and serum calcium and/or PTH.[20,33–35] Whereas a role for calcium in the regulation of FGF-23 cannot be excluded on the basis of our data, the preponderance of the data presented suggest that it is serum 1,25-D rather than serum calcium that is primarily responsible for regulating FGF-23. However, a role for calcium and/or PTH in the regulation of FGF-23 is suggested in patient 3 before surgery (Table 1). However, it is possible that the high FGF-23 levels seen at baseline in patient 3, at a time when serum PTH was very high (863.7 pg/ml), may reflect the ability of PTH to act on FGF-23–producing cells in a synergistic fashion as was suggested in patient 2 (Fig. 2D). As we and others have shown, osteoblasts, which are PTH-responsive cells, produce FGF-23.[9,16,17] The ability to achieve higher FGF-23 levels in the presence of PTH (Fig. 2D) may represent an interesting aspect of FGF-23 physiology and account for the observation that FGF-23 is often elevated in patients with fibrous dysplasia.[9,36] Because fibrous dysplasia is caused by activating $G_s\alpha$ mutations, which mimic activation of the PTH pathway,[37] it is as if the affected osteogenic cells, which have been shown to be a source of FGF-23,[9,16] are hyper-responsive to stimuli that cause FGF-23 synthesis and/or secretion. Even though an activating mutation exists in the cAMP pathway in fibrous dysplasia cells, these cells retain responsiveness to PTH,[38] so it is possible that exposure of fibrous dysplasia cells to PTH could augment 1,25-D–induced FGF-23 production. It is of interest to note that patient 2 required very

high doses of calcitriol and calcium carbonate to obtain eucalcemia. Before admitting this patient, it was suspected that noncompliance with medications accounted for the need to prescribe such high doses. However, during admission, while taking medications under observation, it became apparent she required unusually high doses of calcitriol and calcium carbonate. Normal serum levels of 25-hydroxyvitamin D and vitamins A and E excluded malabsorption as a cause. This raised the possibility that, in the presence of high serum FGF-23, there was resistance to the actions of 1,25-D and invites the speculation that this phenomenon may account for the routinely observed but unexplained need for the high doses of calcitriol that are usually required in the treatment of conditions associated with high FGF-23 (TIO, XLH, ADHR, fibrous dysplasia).

These data show for the first time in humans that serum FGF-23 may be regulated by 1,25-D, that the response of FGF-23–1,25-D may be augmented in the presence of PTH, and in the absence of an adequate PTH effect, the phosphaturic effect of FGF-23 may be diminished.

ACKNOWLEDGMENTS

The authors thank Dr. Pamela Gehron Robey for assistance in the preparation of this manuscript.

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Received in original form March 8, 2005; revised form June 8, 2005; accepted July 13, 2005.